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ORIGINAL RESEARCH

Embryonic fatty acid metabolism in diabetic pregnancy: the difference between embryoblasts and trophoblasts

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ABSTRACT: During the first days of development the preimplantation embryo is supplied with nutrients from the surrounding milieu. Maternal diabetes mellitus affects the uterine microenvironment, leading to a metabolic adaptation processes in the embryo. We analysed embryonic fatty acid (FA) profiles and expression of processing genes in rabbit blastocysts, separately in embryoblasts (EBs) and trophoblasts (TBs), to determine the potential consequences of maternal diabetes mellitus on intracellular FA metabolism. Insulin-dependent diabetes was induced by alloxan in female rabbits. On Day 6 post coitum, FA profiles in blastocysts (EB, TB and blastocoel fluid) and maternal blood were analysed by gas chromatography. The expression levels of molecules involved in FA elongation (fatty acid elongases, ELOVLs) and desaturation (fatty acid desaturases, FADSs) were measured in EB and TB. Maternal diabetes mellitus influenced the FA profile in maternal plasma and blastocysts. Independent from metabolic changes, rabbit blastocysts contained a higher level of saturated fatty acids (SFAs) and a lower level of polyunsaturated fatty acids (PUFAs) compared to the FA profile of the maternal plasma. Furthermore, the FA profile was altered in the EB and TB, differently. While SFAs (palmitic and stearic acid) were elevated in EB of diabetic rabbits, PUFAs, such as docosahexaenoic acid, were decreased. In contrast, in the TB, lower levels of SFAs and higher levels of oleic acid were observed. EB and TB specific alterations in gene expression were found for ELOVLs and FADSs, key enzymes for FA elongation and desaturation. In conclusion, maternal diabetes mellitus alters embryonic FA metabolism differently in EB and TB, indicating a lineage-specific metabolic adaptive response.

Key words: embryoblast / trophoblast / fatty acid metabolism / FADS1 / ELOVL

Introduction

Preimplantation embryo development is a complex, well-orchestrated process, combining dynamic and structural changes during the first days of development. The embryo undergoes important metabolic and structural adaptation processes to ensure the establishment of pregnancy. The nutritional environment *in utero* can have a tremendous effect on embryonic metabolism and it can perturb subsequent development (Leese, 2012). Besides the traditional nutrients, such as glucose, pyruvate and amino acids, also lipids, such as fatty acids (FAs), play a pivotal role in embryonic metabolism (Dunning *et al.*, 2014; Ribeiro *et al.*, 2016).

FAs are chemically classified as saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), whereby PUFAs can be further divided in omega-6 (n-6) and omega-3 (n-3) PUFAs. In animal and human oocytes and embryos, the most common FAs are palmitic acid (PA), stearic acid (SA), oleic acid (OA) and linoleic acid (LA) (Leroy *et al.*, 2005; Haggarty *et al.*, 2006; Dunning *et al.*, 2014). SFAs are preferentially accumulated by the follicle-enclosed oocyte and the early embryo up to the eight-cell stage,

suggesting a selective uptake mechanism or *de novo* synthesis, which favour this class of FAs (Adamiak et al., 2006; Haggarty et al., 2006). PUFAs, such as LA, are predominantly accumulated from the eight-cell stage to the blastocyst stage and onward (Haggarty et al., 2006).

FAs play an important role in fertilization, oocyte maturation and embryo development (Revelli et al., 2009; Dunning et al., 2014; Zarezadeh et al., 2019). While SFAs, PA and SA, have negative effects on fertilization and preimplantation embryo development, the MUFAs OA exerts positive effects (Aardema et al., 2011; McKeegan and Sturmey, 2011; Yang et al., 2017). Controversy exists regarding the role of LA in preimplantation embryo development, with positive effects in humans (Shaaker et al., 2012) and negative effects in ovine and bovine models (Renaville et al., 2010; Amini et al., 2016; Roura et al., 2018). The PUFA α-linolenic acids (ALAs) exerts damaging effects on the reproductive system and it decreases the chance of pregnancy after IVF in humans (Jungheim et al., 2011).

In mammals, FAs have to be provided through the diet or they can be synthesized, up to 16 carbons, via *de novo* lipogenesis. Furthermore, FA desaturation and elongation are two key metabolic routes for the synthesis of long and very long-chain SFAs, MUFAs and PUFAs (reviewed by Zhang et al., 2016). They can be regulated by a wide range of external factors, such as nutrients and hormones. These enzymes have to be well-regulated because they play an important role in cellular lipid composition (Ntambi and Miyazaki, 2004; Jakobsson et al., 2006). Δ5-Desaturase (encoded by FADS1) and Δ6-desaturase (encoded by FADS2) introduce double bonds into PUFAs. FADS2-null mice are sterile (Lattka et al., 2010) and FADS1-ablated mice fail to thrive beyond the 12 weeks of age (Fan et al., 2012). FA elongases can be divided in two major groups: (i) enzymes that are involved in the elongation of SFAs and MUFAs (ELOVL1, ELOVL3 and ELOVL6) and (ii) enzymes which elongate PUFAs (ELOVL2, ELOVL4 and ELOVL5) (Jakobsson et al., 2006). While ELOVL2 and ELOVL5 are predominantly expressed in reproductive organs (Jakobsson et al., 2006; Guillou et al., 2010; Ohno et al., 2010; Zhang et al., 2016), ELOVL1 and ELVOL6 are ubiquitously expressed (Moon et al., 2001; Ohno et al., 2010).

Both desaturation and elongation enzymes are affected in metabolic disorders, such as obesity and diabetes mellitus (Wang et al., 2006). In epidemiological studies, strong associations between Δ5- and Δ6-desaturase activity and a risk of diabetes have been found (Kröger and Schulze, 2012). We recently reported that maternal diabetes mellitus led to maternal hyperlipidaemia accompanied by an elevated intracellular lipid accumulation and altered lipid metabolism in rabbit blastocysts (Schindler et al., 2014). In the current study, we examined how maternal diabetes mellitus influences FA profiles of 6-day-old blastocysts, separately in embryoblast (EB), trophoblast (TB) and blastocysts fluid (BF), compared to maternal serum levels. Furthermore, the expression of genes involved in FA desaturation and elongation was quantified in the rabbit blastocysts to understand how metabolic pathways are regulated. For this purpose, a chemically-induced insulin-dependent diabetic rabbit model was used. Rabbits have been used as animal models for early embryo development (Fischer et al., 2012) and lipid metabolism for a long time. Rabbit lipid metabolism is more closely related to human metabolism than mice or rat metabolism (Paigen, 1995). Data from human and rabbit blastocysts indicate a very similar proportion of SFAs, MUFAs and PUFAs (Khandoker et al., 1998; Haggarty et al., 2006), further supporting the fact that rabbit preimplantation embryo development is an excellent model for analysing embryonic FA metabolism in detail. Overall, this study further supports the findings that maternal diabetes mellitus affects embryonic lipid metabolism, leading to altered adaptation processes during preimplantation development.

Methods

Alloxan treatment

Experimental insulin-dependent diabetes (DT1) was induced in mature 18- to 20-week-old female non-pregnant rabbits (outbred ZIKA-hybrid New Zealand White) by alloxan treatment (Sigma-Aldrich, Germany) as described by Ramin et al. (2010). Rabbits were maintained under diabetic conditions (blood glucose levels 15–25 mmol/l, daily insulin supplementation) as described by Schindler et al. (2017) for at least 10 days before mating. All animal experiments were performed in accordance with the principles of laboratory animal care and the experimental protocol had been approved by the local ethics committee (Landesverwaltungsamt Dessau; reference number: 42502-2-812).

Embryo recovery

Embryos were collected from diabetic (DT1) and non-diabetic (NI) rabbits at Day 6 post coitum (p.c.). Mating and embryo recovery were performed as described by Schindler et al. (2013). Six-day-old (d6) blastocysts were characterized morphologically and grouped by gastrulation stages. For analysis, gastrulation stages 1 and 2 were used (summarized by Fischer et al., 2012).

For FA analysis, flushed blastocysts were washed three times with ice cold phosphate buffered saline (PBS). The blastocyst stage and size were determined. Blastocysts were then placed on a dry watch glass. Extracellular coverings were removed mechanically, blastocysts were punctured and the effluent BF was collected. Blastocysts were then mechanically dissected with surgical forceps and scissors into TB and EB (working scheme: Supplementary Fig. S1). Individual samples of EB, TB and BF were stored separately at −80°C for further FA analysis, as described below. For RNA analyses, samples of EB and TB were stored in PBS at −80°C until RNA isolation for quantitative polymerase chain reaction (RT-qPCR). For western blot analyses, embryos were handled in 0.05% polyvinyl alcohol (PVA)/PBS containing protease and phosphatase inhibitors. For one protein sample, a minimum of eight EB or TB from three different rabbits were pooled in one tube. Samples were stored in radio immunoprecipitation assay buffer with protease and phosphatase inhibitors at −80°C until further processing.

Lipid extraction and FA analysis

Lipid extraction was performed in single embryos (BF, EB and TB; per experimental group (NI vs. DT1) samples from 20 embryos have been used) and maternal plasma by adding up to 8 ml of chloroform/methanol (2:1, v/v) dropwise at room temperature. The solution contained C19:0 (22.5 µg) as an internal standard. The detailed sample preparation procedure was previously described by Dannenberger et al. (2017). In this procedure, the extraction mixtures were stirred twice for 15 min, stored at 5°C for 18 h in the dark and subsequently

washed with 0.02% $CaCl_2$ solution. Finally, the solvent was removed under a gentle nitrogen stream at room temperature. The lipid extracts were re-dissolved in 150 µl of toluene for methyl ester preparation. Next, 1 ml of 0.5 M sodium methoxide in methanol was added to the samples, which were shaken in a 60°C water bath for 10 min. Subsequently, 0.5 ml of 14% boron trifluoride (BF_3) in methanol was added to the mixture, which was then shaken for an additional 10 min at 60°C. The FA methyl esters (FAMEs) were extracted three times in 2 ml of *n*-hexane. The FAMEs were resuspended in 100 µl of *n*-hexane and stored at −18°C until gas chromatography (GC) analysis.

FA analysis of the different cell compartments (EB, TB and BF) was performed using capillary GC with a CP-Sil 88 CB column (100 m × 0.25 mm, Chrompack-Varian, Lake Forest, CA, USA) that was installed in a PerkinElmer gas chromatograph (CLARUS 680) with a flame ionisation detector and split injection (PerkinElmer Instruments, Shelton, USA). The detailed GC conditions were recently described by Dannenberger *et al.* (2017). The FA profiling was done with the use of C19:0 as an internal standard. For the calibration procedure, the reference standard mixture 'Sigma FAME' (Sigma-Aldrich, Deisenhofen, Germany) and the methyl esters of C18:1*cis*-11, C22:5*n*-3, C18:2*cis*-9, *trans*-11 (Matreya, PA, USA), C22:4*n*-6 (Sigma-Aldrich, Deisenhofen, Germany) and C18:4*n*-3 (Larodan, Limhamn, Sweden) were used. The results are expressed as relative amounts (%) of all measured FAs in the embryonic samples (EB, TB, and BF) and the maternal plasma.

RNA isolation and cDNA synthesis

Messenger RNA (mRNA) of single blastocysts, separated in EB and TB, was extracted with Dynabeads® Oligo $(dT)_{25}$ (Invitrogen, Germany) and used for cDNA synthesis. All protocol procedures were carried out according to the manufacturer's instructions, using the modifications previously described (Schindler *et al.*, 2013). The final volume of the cDNA reaction was adjusted with water to 80 µl.

Polymerase chain reaction (RT-PCR)

RT-PCR amplification was carried out with 0.5 µl of cDNA from single blastocysts in 25 µl containing 200 µM of dNTP, 2.5 U Taq polymerases, specific oligonucleotides for FADSs, ELOVLs and GAPDH (primers listed in Table 1). The amplification was done for 40 cycles (94°C 45 s, 60°C 45 s, 72°C 60 s). The resulting PCR products were separated by electrophoresis on 2% agarose gel and stained with ethidium bromide.

Quantitative polymerase chain reaction (RT-qPCR)

Quantitative polymerase chain reaction (RT-qPCR) was performed in duplicate using the Applied Biosystems StepOnePlus™ System (Applied Biosystems, Germany), with no template control for each primer set with SYBR Green detection. The nucleotide sequences of the primers used in this study are listed in Table 1. The PCR products were sequenced and analysed as described (Schindler *et al.*, 2013). Sequence homology was proven by using the alignment BLASTN tool. GAPDH was simultaneously quantified as the endogenous control and the target gene expression was normalized to that of GAPDH in each sample. Simultaneously, GAPDH mRNA levels were quantified as the endogenous reference. The target gene expression was normalized to that of GAPDH in each sample. To prove that GAPDH expression was unaffected by the treatment, the amount of GAPDH was compared to total amount of mRNA and vinculin transcription. Equal quantities of GAPDH mRNA were observed in embryonic samples from diabetic (DT1) and normoinsulinaemic (NI) rabbits, whereas the GAPDH expression level was different in EB and TB (Supplementary Fig. S2). In each RT-qPCR, a calibration curve was included from serial dilutions in the range of 10^7 to 10^3 copies of primer-specific DNA plasmid standards. Results were calculated as amounts of target mRNA in molecules per molecules GAPDH mRNA and expressed as relative amounts in percentages of the control samples.

Protein preparation and immunoblotting

Protein preparation, quantification and western blot were performed with EB and TB samples from 8 to 10 blastocysts, as described by Schindler *et al.* (2013). For western blot analysis, 25 µg of total protein lysates were subjected to SDS-polyacrylamide electrophoresis and electro-transferred to nitrocellulose membranes. For detection of FADS1, FADS2 and β-actin, membranes were blocked in Tris-buffered saline containing 0.1% Tween with 3% (wt/vol) non-fat dry milk at room temperature for at least 1 h. The primary antibody was incubated at 4°C overnight. Antibodies were purchased from FADS1 (#sc-134337, Santa Cruz, Germany, 1:1000), FADS2 (#AP22270a, Abgent, Germany, 1:1000), β-actin (#A5441Sigma-Aldrich, Germany, 1:40 000) and anti-mouse IgG conjugated to HRP (Dianova, Germany, 1:20 000). A summary of the antibodies used in this study are listed in Table 2. Protein was detected using Luminata Forte (Milipore) imaged with Chemi Doc ImageLab Software (BioRad). The amount of protein was calculated as the ratio of band intensities (FADS1 and FADS2 protein vs. β-actin) in the same blot to correct for differences in protein loading.

Statistics

FA data were analysed with the software package SAS (SAS© Systems, Release 9.4, SAS Institute Inc., Cary, NC, USA). These data were modelled with a two-way repeated measurement ANOVA, with fixed factor group (normoinsulinaemic and diabetic rabbits), and repeated factor embryonic compartment (blastocyst cavity fluid, EB, TB). Tests of least square means were adjusted for multiple testing by Tukey–Kramer correction. Statistical significances are indicated if $P \leq 0.05$.

Protein and RNA data are expressed as mean value ± SEM. Levels of significance between groups were calculated using the Student's *t*-test after proving normal distribution. Multiple comparisons were made by factorial variance analysis (ANOVA) adjusted according to Bonferroni (SigmaPlot v. 12.0). Statistical significances are indicated as follows $^*P < 0.05$. All experiments were repeated at least three times.

Results

FA composition in the embryoblast, trophoblast and blastocysts cavity fluid

The FA profile was analysed separately in embryonic compartments: EB, TB and BF (Table 3). Rabbit blastocysts contained high amounts of SFAs (sum SFA, with 76–78% of the total FAs per compartment). The

Table I Primers used for RT-PCR and RT-qPCR.

Gene name	Genbank number	T (°C)	Fragment (bp)	Sequence 5′→3′
FADS1	XM_008274405.2	60	101	fw: AGGAAGGACACGAGTTCAGG rev: CAATCGTCTCCAAGCCACTG
FADS2	NM_001329067.1	60	185	fw: AGCACCACCTCTTTCCTACC rev: TCATTTGTGGAGGTAGGCGT
ELOVL1	XM_008265411.2	60	151	fw: ACTTTGTCCTCTCACTGGGG rev: GTATAAGTGCTCAGCCAGCCA
ELOVL2	XM_002714176	60	100	fw: GGATACCGTGCGGACAGAG rev: TGCATGGACGGAAACACAGA
ELOVL5	XM_002714509	60	110	fw: CTCCCGAAGGAAAGACCACC rev: GCTTCCGTGGCTTCACATTG
ELOVL6	XM_002717122.3	60	147	fw: GCTCTGGTCTCTGACCCTTG rev: GCCCAGAATTTGCTGACTGG
GAPDH	L23961	60	144	fw: GCCGCTTCTTCTCGTGCAG rev: ATGGATCATTGATGGCGACAACAT

Table II List of primary and secondary antibodies used in western blotting.

Antigen	Name of antibody	Manufacturer/company # catalogue	Type	Dilution
FADS1	FADS1 antibody (7-RY13)	Santa cruz, #sc-134337	MM	1:1000
FADS2	FADS2 antibody	Abgent, #AP22270a	MM	1:1000
β-actin	Anti-β-actin antibody clone AC-15	Sigma, #A5441	MM	1:40 000
Mouse IgG	Goat anti-mouse IgG (H+L)-HRPO	Dianova, #115-036-003	GP	1:20 000

GP, goat polyclonal; MM, mouse monoclonal.

most abundant FA was PA (16:0), followed by SA (18:0) and OA (18:1cis-9). Compared to EB and BF, TB cells had the highest PA and SA level with around 40% and 21% of the total FAs, respectively. The relative OA (18:1cis-9) level was slightly lower in TB, compared to EB and BF. Furthermore, TB cells contained a 4-fold and 10-fold higher amount of LA (18:2n-6) and arachidonic acid (AA) (20:4n-6) compared to EB and BF, respectively. The amount of both n-3 FAs, docosapentaenic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) was significantly lower (5- to 7-fold) in the TB compared to EB and BF. In summary, the FA profile in the BF was similar to the FA composition in EB cells, but it differed from the TB.

FA composition in the preimplantation embryo from diabetic rabbits

The amounts of FAs in EB, TB, and BF from diabetic rabbits and normoinsulinaemic control rabbits are compared in Table 3. The amounts of all SFAs measured in our study (sum SFA in Table 3) were not affected in the EB and BF. However, the relative amounts of individual SFAs, PA (16:0) and SA (18:0) were increased in the EB cells and BF due to maternal diabetes mellitus. Other SFAs were either not affected or decreased in BF and EB.

In the TB from diabetic rabbits, a significant lower amount of SFA was observed. In TB samples from normoinsulinaemic rabbits (NI), 76.32% of all FA were SFA, and in diabetic (DT1) TB samples, only 60.64% of all FA belonged to the SFA. This decrease was mainly a consequence of lower levels of PA and SA (PA: NI: 40.17% and DT1: 30.00%; SA: NI: 21.36% and DT1: 17.95%). Other medium-chain or long-chain FAs were also decreased, while very long-chain FAs (21 carbon atoms and longer) were increased in TB cells from diabetic rabbits.

The amount of MUFAs (sum MUFA, Table 3) was elevated in TB cells (1.6-fold increase) from diabetic rabbits, which was mainly due to a higher amount of OA (18:1cis-9).

In blastocyst from diabetic rabbits, the total amount of PUFAs (sum PUFA, Table 3) was not changed in EB and BF samples due to a diabetes mellitus, but in TB cells, it was increased by a 1.7-fold. To be more precise, n-3 and n-6 FAs were examined individually. Total amounts of n-6 FAs (sum n-6 FA, Table 3) were increased in all investigated blastocysts compartments, while the sums of n-3 (sum n-3 FA, Table 3) FAs were greatly decreased in EB and TB (by 85% and 80%,

Table III Relative amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) investigated in rabbit blastocysts.

Fatty acids	Relative amount (% of total amount)						Overall effects	
	Blastocyst cavity fluid		Embryoblast		Trophoblast			
	NI	DT1	NI	DT1	NI	DT1	Diabetes	Cell compartment
	n = 19	n = 20	n = 20	n = 20	n = 20	n = 20	*P*-value	*P*-value
SFA								
12:0 (Lauric acid)	$1.83^{a}_{0.16}$	$1.30^{a}_{0.16}$	$2.29^{b.a}_{0.16}$	$1.25^{a}_{0.16}$	$1.60^{a}_{0.16}$	$0.68^{b}_{0.16}$	0.0017	<0.0001
13:0 (Tridecyclic acid)	$0.27^{a}_{0.02}$	$0.28^{a}_{0.02}$	$0.23_{0.02}$	$0.28^{a}_{0.03}$	$0.11_{0.10}$	$0.17^{b}_{0.02}$	0.3386	0.0366
14:0 (Myristic acid)	$5.63^{a}_{0.16}$	$3.06^{b.a}_{0.16}$	$4.57^{b}_{0.16}$	$3.59^{b.a}_{0.16}$	$3.70^{b.a}_{0.16}$	$2.23^{b}_{0.16}$	<0.0001	<0.0001
15:0 (Pentadecylic acid)	$3.22^{a}_{0.10}$	$1.45^{b.a}_{0.10}$	$2.33^{b}_{0.10}$	$1.96^{b}_{0.10}$	$1.72^{b}_{0.10}$	$1.16^{b.a}_{0.10}$	<0.0001	<0.0001
16:0 (Palmitic acid)	$36.05^{a}_{0.65}$	$40.54^{b.a}_{0.64}$	$33.51^{b}_{0.64}$	$41.02^{b.a}_{0.64}$	$40.17^{b.a}_{0.64}$	$30.00^{b}_{0.64}$	0.4091	<0.0001
17:0 (Margaric acid)	$1.65_{0.20}$	$1.91_{0.20}$	$1.55_{0.20}$	$2.42^{a}_{0.20}$	$2.10^{a}_{0.20}$	$1.21^{b}_{0.20}$	0.6251	0.2437
18:0 (Stearic acid)	$13.86^{a}_{0.68}$	$25.17^{b.a}_{0.66}$	$15.09^{a}_{0.66}$	$21.96^{b.a}_{0.66}$	$21.36^{b.a}_{0.66}$	$17.95^{b}_{0.66}$	<0.0001	0.1165
20:0 (Arachidic acid)	$2.15^{a}_{0.04}$	$1.10^{b}_{0.04}$	$2.08^{a}_{0.04}$	$0.89^{b.a}_{0.04}$	$1.07^{b.a}_{0.04}$	$0.94^{b.a}_{0.04}$	<0.0001	<0.0001
21:0 (Heneicosylic acid)	$1.21^{a}_{0.03}$	$0.27^{b}_{0.03}$	$1.20^{a}_{0.03}$	$0.22^{b}_{0.03}$	$0.26^{b}_{0.03}$	$0.46^{b.a}_{0.03}$	<0.0001	<0.0001
22:0 (Behenic acid)	$4.34^{a}_{0.11}$	$1.38^{b}_{0.11}$	$4.40^{a}_{0.11}$	$1.43^{b}_{0.11}$	$1.59^{b}_{0.11}$	$1.85^{b.a}_{0.11}$	<0.0001	<0.0001
23:0 (Tricosylic acid)	$1.74^{a}_{0.09}$	$0.42^{b}_{0.09}$	$1.77^{a}_{0.09}$	$0.62^{b}_{0.13}$	$0.53^{b}_{0.09}$	$0.75^{b}_{0.09}$	<0.0001	<0.0001
24:0 (Lignoceric acid)	$6.02^{a}_{0.13}$	$1.26^{b.a}_{0.13}$	$5.78^{a}_{0.13}$	$0.90^{b.a}_{0.13}$	$1.23^{b.a}_{0.13}$	$2.67^{b}_{0.13}$	<0.0001	<0.0001
Sum SFA	$78.14^{a}_{1.03}$	$78.80^{a}_{1.01}$	$75.21^{a}_{1.01}$	$77.19^{a}_{1.01}$	$76.32^{a}_{1.01}$	$60.64^{b}_{1.01}$	0.0015	<0.0001
MUFA								
16:1*c*-9 (Palmitoleic acid)	$0.57^{a}_{0.25}$	$0.99^{a}_{0.21}$	$0.55^{a}_{0.23}$	$3.02^{b}_{0.21}$	$1.04^{a}_{0.23}$	$2.38^{b}_{0.21}$	0.0425	<0.0001
17:1*c*-10 (cis-10-Heptadecenoic acid)	$0.25_{0.26}$	$0.26_{0.08}$	n.d.	n.d.	$0.27_{0.10}$	$0.21_{0.05}$	0.8922	0.4908
18:1*c*-9 (Oleic acid)	$10.49^{a}_{0.73}$	$9.47^{a}_{0.72}$	$12.20^{a}_{0.72}$	$11.46^{a}_{0.71}$	$9.63^{b.a}_{0.72}$	$20.64^{b}_{0.71}$	0.0023	<0.0001
18:1*c*-11 (cis-Vaccenic)	$0.46^{a}_{0.07}$	$0.73^{a}_{0.07}$	$0.57^{a}_{0.07}$	$0.88^{b}_{0.07}$	$0.82^{b}_{0.07}$	$1.70^{b.a}_{0.07}$	<0.0001	<0.0001
18:1*t*-9 (Elaidic acid)	$0.46^{a}_{0.05}$	$0.11^{b}_{0.07}$	$0.60^{a}_{0.05}$	$0.30^{b}_{0.06}$	n.d.	n.d.	<0.0001	0.0215
20:1*c*-11 (Gondoic acid)	$0.15^{a}_{0.03}$	$0.31^{b.a}_{0.03}$	$0.18^{a}_{0.03}$	$0.32^{b.a}_{0.03}$	$0.24^{b.a}_{0.03}$	$0.55^{b}_{0.03}$	0.0002	<0.0001
22:1*c*-13 (Erucic acid)	$0.08^{a}_{0.02}$	$0.11^{a}_{0.01}$	$0.02^{b}_{0.02}$	n.d.	n.d.	$0.07^{b}_{0.01}$	0.1389	0.0049
24:1*c*-15 (Nervonic acid)	$0.84^{a}_{0.14}$	$1.63^{b}_{0.15}$	$1.36^{b}_{0.14}$	$0.44^{a}_{0.18}$	$4.23^{b}_{0.14}$	$0.25^{a}_{0.15}$	<0.0001	<0.0001
Sum MUFA	$13.02^{a}_{0.83}$	$13.06^{a}_{0.81}$	$15.46^{b.a}_{0.81}$	$16.34^{b.a}_{0.81}$	$16.00^{b.a}_{0.81}$	$26.09^{b}_{0.81}$	0.0013	<0.0001
PUFA								
18:2*n*-6 (Linoleic acid)	$0.52^{a}_{0.26}$	$2.73^{b.a}_{0.25}$	$0.54^{a}_{0.25}$	$2.40^{b.a}_{0.25}$	$2.05^{b.a}_{0.26}$	$6.60^{b}_{0.25}$	<0.0001	<0.0001
18:2*t*-9,*t*-11(t9,t11-Octadecadienoic acid)	$0.22^{a}_{0.22}$	$0.19^{a}_{0.21}$	$0.21^{a}_{0.21}$	$2.00^{b}_{0.25}$	$1.02^{b.a}_{0.21}$	$0.29^{a}_{0.22}$	0.1794	<0.0001
18:3*n*-3 (α-Linolenic acid)	$0.19^{a}_{0.05}$	$0.34^{a}_{0.04}$	$0.20^{a}_{0.14}$	$0.42^{b.a}_{0.05}$	$0.31^{a}_{0.05}$	$0.64^{b}_{0.03}$	0.0003	<0.0001
18:3*n*-6 (γ-Linolenic acid)	$0.11^{a}_{0.11}$	$0.39^{a.b}_{0.10}$	$0.09^{a}_{0.12}$	$0.46^{b}_{0.12}$	$0.10^{a}_{0.15}$	$0.07^{a}_{0.11}$	0.0487	0.2951
20:2*n*-6 (Eicosadienoic acid)	$0.44^{a}_{0.03}$	$0.17^{b}_{0.04}$	$0.46^{a}_{0.03}$	$0.17^{b}_{0.06}$	$0.22^{b}_{0.04}$	$0.38^{a}_{0.03}$	0.0050	0.9501
20:3*n*-6 (Dihomo-γ-linolenic acid)	$0.10^{a}_{0.13}$	$0.87^{b}_{0.10}$	$0.15^{a}_{0.11}$	$0.85^{b}_{0.17}$	$0.41^{b.a}_{0.11}$	$0.48^{b.a}_{0.09}$	0.0015	0.8714
20:3*n*-3 (Eicosatrienoic acid)	$0.11^{a}_{0.03}$	$0.11^{a}_{0.02}$	$0.09^{a}_{0.03}$	$0.43^{b}_{0.07}$	n.d.	$0.13^{a}_{0.02}$	0.0005	0.0012
20:4*n*-6 (Arachidonic acid)	$0.23^{a}_{0.39}$	$2.25^{b}_{0.28}$	$0.16^{a}_{0.38}$	$0.72^{a}_{0.46}$	$1.96^{b}_{0.27}$	$1.59^{b}_{0.27}$	0.0674	<0.0001
20:5*n*-3 (Eicosapentaenoic acid)	$0.05^{a}_{0.10}$	$0.16^{a}_{0.07}$	n.d.	$0.19^{a}_{0.06}$	n.d.	$0.09^{a}_{0.03}$	0.3646	0.3975
22:2*n*-6 (Docosadienoic acid)	$0.25^{a}_{0.09}$	$0.41^{a.b}_{0.09}$	$0.18^{a}_{0.09}$	$0.68^{b.a}_{0.11}$	$0.41^{a.b}_{0.09}$	$0.41^{a.b}_{0.09}$	0.0179	0.5484
22:5*n*-3 (Docosapentaenoic acid)	$4.46^{a}_{0.12}$	$0.56^{b}_{0.12}$	$4.43^{a}_{0.12}$	$0.37^{b}_{0.13}$	$0.63^{b}_{0.12}$	$1.98^{b.a}_{0.12}$	<0.0001	<0.0001
22:6*n*-3 (Docosahexaenoic acid)	$2.46^{a}_{0.10}$	$0.54^{b}_{0.10}$	$3.00^{b.a}_{0.10}$	$0.55^{b}_{0.11}$	$0.58^{b}_{0.10}$	$0.43^{b}_{0.10}$	<0.0001	<0.0001
Sum PUFA	$8.95^{a}_{0.56}$	$8.09^{a}_{0.54}$	$9.34^{a}_{0.54}$	$6.47^{b}_{0.54}$	$7.73^{a}_{0.54}$	$13.27^{b}_{0.54}$	0.0007	<0.0001
Sum n-3 FA	$7.02^{a}_{0.19}$	$1.44^{b.a}_{0.19}$	$7.46^{a}_{0.19}$	$1.11^{b.a}_{0.19}$	$1.35^{b.a}_{0.19}$	$3.26^{b.a}_{0.19}$	<0.0001	<0.0001
Sum n-6 FA	$1.60^{a}_{0.35}$	$6.48^{b}_{0.34}$	$1.65^{a}_{0.34}$	$4.04^{b.a}_{0.34}$	$5.30^{b.a}_{0.34}$	$9.74^{b.a}_{0.34}$	<0.0001	<0.0001
Ratio n-6/n-3	$0.24^{a}_{0.35}$	$4.80^{b.a}_{0.35}$	$0.24^{a}_{0.35}$	$4.34^{b.a}_{0.35}$	$4.39^{b}_{0.35}$	$3.12^{b}_{0.35}$	0.0001	<0.0001

Different letters denote significant effect of group (a, b) ($P \leq 0.05$).
The amount was calculated as mean ± SEM ($Mean_{SEM}$) in percent of the total fatty acid content in the embryonic compartments.
DT1, experimental insulin-dependent diabetic; n, number of embryos pooled from 3 to 4 animals per experimental group; n.d., not detectable; NI, normoinsulinaemic rabbits.

respectively) from diabetic rabbits. No changes were observed in the TB. The ratio of n-6 to n-3 FAs (line ratio n-6/n-3, Table 3) was highly elevated in BF and EB samples from diabetic rabbits with an almost a 20-fold increase and this ratio was found to be slightly decreased in TB samples. DHA (22:6n-3) and DPA (22:5n-3) were the n-3 PUFAs with the highest occurrence in EB and BF in a healthy control rabbit. Due to maternal diabetes mellitus, the amount of DHA and DPA decreased by 78% to 92%, respectively (Table 3). Furthermore, there was 2-fold increase in ALA (18:3n-3) in EB and TB compartment. The n-6 FAs LA (18:2n-6) was highly increased (3.3-fold in TB, 4.4-fold in EB and 5.3-fold in BF) in the rabbit blastocyst. The n-6 FA AA (20:4n-6) was increased 10-fold in BF, while no significant changes were observed in the EB and TB (Table 3).

FA profile in the maternal plasma of rabbits

In addition to embryonic FA levels, we measured FA levels in maternal plasma samples from diabetic and non-diabetic pregnant rabbits. The ratio of SFAs, MUFAs and PUFAs in the plasma of the mother (Table 4) was clearly different compared to the blastocysts (Table 3). The amount of SFAs (sum SFA, maternal plasma: Table 4, blastocysts: Table 3) was lower in comparison to the relative amounts in embryonic tissues, which was due to a lower level of PA (16:0) and very long-chain FAs (21 carbon atoms and longer). The total amounts of MUFAs and PUFAs (sum MUFA and PUFA, blastocysts: Table 3 and maternal plasma: Table 4) were higher in maternal plasma samples than in the rabbit blastocysts, in particular, the amount of LA (18:2n-6) increased 10- to 40-fold.

In diabetic rabbits, the total amount of SFAs decreased in maternal plasma. Analysis of the different SFAs revealed that some of them were either not affected or decreased (e.g. PA, 16:0 and SA, 18:0, decreased by 25%). The total amount of MUFAs was lower in the plasma from diabetic rabbits, whereby the most prominent monounsaturated fatty OA (18:1cis-9) acid did not change.

The sum of the PUFAs increased in plasma from diabetic rabbits with marked differences found between the individual PUFAs. While the relative amounts of LA (18:2n-6) and ALA (18:3n-3) increased (20% and 220%, respectively), other PUFAs, such as AA (20:4n-6), eicosapentaenoic acid (20:5n-3) and DHA (22:6n-3), decreased. Furthermore, the ratio of n-6 vs. n-3 PUFAs was lower in rabbit plasma with experimentally-induced diabetes mellitus.

Expression of enzymes for FA desaturation and elongation in the rabbit preimplantation embryo

In the next step, we evaluated whether or not metabolic changes due to maternal diabetes could affect key enzymes for FA synthesis and processing in the blastocysts. Therefore, the expression of the genes involved in FA elongation and desaturation was verified by RT-PCR in the rabbit preimplantation embryo (Fig. 1). FA desaturases (FADS1 and FADS2) and elongases (ELOVL1, ELOVL2, ELOVL5 and ELOVL6), were expressed from morulae stages (Day 3 p.c.) till the late blastocysts stage (Day 8 p.c.) (Fig. 1). We observed transcription of all investigated genes during preimplantation development and in both blastocysts compartments, EB and TB (Fig. 1).

In blastocysts from diabetic rabbits, mRNA and protein amount of FADS1 were increased in the TB, whereas no changes were observed in the EB (Fig. 2A and B). In contrast, mRNA and protein of FADS2 was increased only in the EB and not affected in the TB from diabetic rabbits (Fig. 2C and D).

To estimate the enzyme activity, the desaturase index was calculated as the ratio of FA product to precursor (Kröger and Schulze, 2012). Δ5-Desaturases activity was expressed as the ratio of AA (20:4n-6) to dihomo-γ-linolenic acid (20:3n-6), while Δ6-desaturases activity was estimated as the ratio of γ-linolenic acid (18:3n-6) to LA (18:2n-6) (Table 5). The calculated Δ5-desaturases activity was higher in TB than in EB. Furthermore, the Δ5-desaturases activity was lower in TB from diabetic rabbits. The Δ6-desaturases activity was not affected in EB and TB by maternal diabetes.

ELOVLs activity is known to be controlled by transcriptional regulation (Jakobsson et al., 2006). Therefore, the expression of ELOVL1, ELOVL2, ELVOL5 and ELOVL6 was quantified by RT-qPCR (Fig. 3). The mRNA amounts of ELOVL1 and ELOVL2 increased in EB and TB from diabetic rabbits (Fig. 3A and B), while no significant differences were observed for ELOVL5 and 6 (Fig. 3C and D).

Discussion

FAs are key nutrients critical for growth and development, serving as substrates for signalling molecules and acting as direct modulators of gene expression (Turner et al., 2014). Previous research has revealed the importance of FAs for the early mammalian embryo (Hillman and Flynn, 1980). On the one hand, FAs serve as energy sources during preimplantation development. Rabbit embryos oxidize PA at the single-cell stage, with a subsequent increase during further embryo development. The highest rate of PA oxidation has been measured at the blastocyst stage. On the other hand, in rabbit zygotes, FAs taken up from the surrounding milieu can also be incorporated in triacylglycerols and phospholipids for energy storage (Waterman and Wall, 1988). In the current study, we observed that the FA composition was different in EB and TB compartments and that the FA composition of BF was closer to EB than to TB. Since EB and TB develop in the same surrounding milieu, it is likely that EB and TB take up different FAs to cover their needs.

Compared to the maternal plasma FA profile, rabbit preimplantation embryos contain higher amounts of SFAs and lower amounts of PUFAs, especially n-6 PUFAs. The reason for difference in FA composition between embryo and the mother is not fully understood. It has been reported that changes in serum FAs are reflected in the FA composition of the follicular environment (Childs et al., 2008; Fouladi-Nashta et al., 2009). A recent study in humans showed a positive correlation between the follicular fluid and the blood serum concentration of PUFAs, such as ALA and LA. No correlation has been observed for SFAs, such as PA and SA (Lash and Armstrong, 2009; Mirabi et al., 2017). Data concerning the microenvironment within the uterus are less well established. Haggerty and co-workers showed that human embryos favour SFA accumulation in the early preimplantation phase up to the eight-cell stage. From the eight-cell stage to the blastocysts stage and onward, PUFAs are predominately accumulated (Haggarty et al., 2006). Also, a study in rabbits has shown that the fatty acid composition change in embryos from one-cell to blastocysts stage and

Table IV Relative amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in blood plasma of normoinsulinaemic and diabetic rabbits at Day 6 p.c. (Mean$_{SEM}$).

Fatty acids	Relative amount (% of total amount)	
	Normoinsulinaemic n = 19	Diabetes type 1 n = 20
SFA		
12:0 (Lauric acid)	$0.63^{a}_{0.04}$	$0.38^{b}_{0.04}$
13:0 (Tridecyclic acid)	$0.08_{0.01}$	$0.06_{0.01}$
14:0 (Myristic acid)	$1.50^{a}_{0.06}$	$1.26^{b}_{0.05}$
15:0 (Pentadecylic acid)	$0.76_{0.02}$	$0.73_{0.02}$
16:0 (Palmitic acid)	$23.15_{0.23}$	$23.12_{0.23}$
17:0 (Margaric acid)	$0.85_{0.02}$	$0.78_{0.02}$
18:0 (Stearic acid)	$12.62^{a}_{0.22}$	$9.60^{b}_{0.22}$
20:0 (Arachidic acid)	$0.41_{0.06}$	$0.35_{0.06}$
21:0 (Heneicosylic acid)	$0.18^{a}_{0.02}$	$0.11^{b}_{0.02}$
22:0 (Behenic acid)	$0.75^{a}_{0.06}$	$0.39^{b}_{0.06}$
23:0 (Tricosylic acid)	$0.29_{0.04}$	$0.27_{0.04}$
24:0 (Lignoceric acid)	$0.93^{a}_{0.10}$	$0.51^{b}_{0.10}$
Sum SFA	$42.36^{a}_{0.43}$	$37.61^{b}_{0.42}$
MUFA		
16:1cis-9 (Palmitoleic acid)	$1.29^{a}_{0.08}$	$1.71^{b}_{0.08}$
17:1cis-10 (cis-10-Heptadecenoic acid)	$0.39^{a}_{0.05}$	$0.26^{b}_{0.04}$
18:1cis-9 (Oleic acid)	$17.88_{0.30}$	$17.34_{0.30}$
18:1cis-11 (cis-Vaccenic)	$1.24^{a}_{0.04}$	$1.06^{b}_{0.04}$
18:1trans-9 (Elaidic acid)	$0.16^{a}_{0.01}$	$0.08^{b}_{0.01}$
18:1trans-11 (trans-vaccenic acid)	$0.08^{a}_{0.001}$	$0.10^{b}_{0.01}$
20:1*c*-11 (Gondoic acid)	$0.25^{a}_{0.01}$	$0.17^{b}_{0.01}$
22:1*c*-13 (Erucic acid)	$0.03^{a}_{0.001}$	$0.02^{b}_{0.001}$
24:1*c*-15 (Nervonic acid)	$0.49^{a}_{0.03}$	$0.04^{b}_{0.04}$
Sum MUFA	$21.86^{a}_{0.39}$	$20.78^{b}_{0.38}$
PUFA		
18:2*n*-6 (Linoleic acid)	$26.60^{a}_{0.40}$	$31.79^{b}_{0.39}$
18:2*t*-9,*t*-11(t9, t11-Octadecadienoic acid)	$0.23^{a}_{0.02}$	$0.14^{b}_{0.02}$
18:3*n*-3 (α-Linolenic acid)	$2.11^{a}_{0.14}$	$4.72^{b}_{0.14}$
18:3*n*-6 (γ-Linolenic acid)	$0.05^{a}_{0.03}$	$0.14^{b}_{0.03}$
18:4n-3 (Stearidonic acid)	$0.005_{0.005}$	$0.01_{0.002}$
20:2*n*-6 (Eicosadienoic acid)	$0.40^{a}_{0.03}$	$0.23^{b}_{0.03}$
20:3*n*-6 (Dihomo-γ-linolenic acid)	$0.42^{a}_{0.01}$	$0.12^{b}_{0.01}$
20:3*n*-3 (Eicosatrienoic acid)	$0.06_{0.02}$	$0.08_{0.02}$
20:4*n*-6 (Arachidonic acid)	$4.09^{a}_{0.13}$	$3.30^{b}_{0.12}$
20:5*n*-3 (Eicosapentaenoic acid)	$0.15^{a}_{0.01}$	$0.10^{b}_{0.01}$
22:2*n*-6 (Docosadienoic acid)	$0.07_{0.01}$	$0.06_{0.01}$
22:5*n*-3 (Docosapentaenoic Acid)	$0.79^{a}_{0.07}$	$0.45^{b}_{0.07}$
22:6*n*-3 (Docosahexaenoic acid)	$0.43^{a}_{0.04}$	$0.14^{b}_{0.04}$
Sum PUFA	$35.74^{a}_{0.50}$	$41.56^{b}_{0.49}$
Sum n-3 PUFA	$3.54^{a}_{0.16}$	$5.49^{b}_{0.15}$
Sum n-6 PUFA	$31.97^{a}_{0.44}$	$35.96^{b}_{0.43}$
Ratio n-6/n-3 PUFA	$9.63^{a}_{0.33}$	$6.74^{b}_{0.32}$

Different letters denote significant effect of group (a, b) ($P \leq 0.05$).

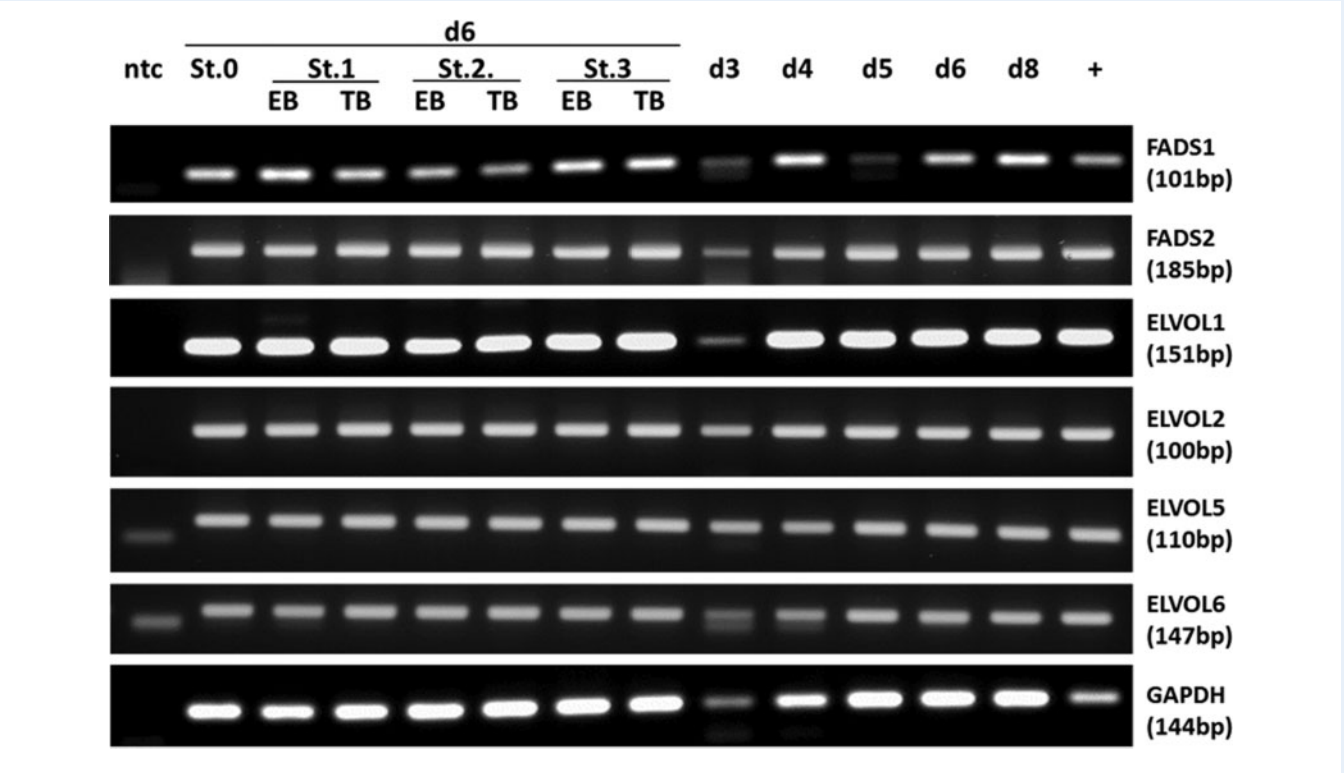

Figure 1. Transcription of key genes in fatty acid metabolism in rabbit preimplantation embryos. RT-PCR was performed with specific primers for FADS1, FADS2, ELOVL1, ELOVL2, ELOVL5 and ELVOL6 on rabbit morulae at Day (d) 3, blastocysts at Days 4, 5, 6 and 8 post coitum; and d6 blastocysts at gastrulation stages (st.) 0, 1, 2 and 3. Gastrulating blastocysts were separated in embryoblast (EB) and trophoblast (TB). Transcripts of FADS1, FADS2, ELOVL1, ELOVL2, ELOVL5 and ELOVL6 were detected in all analysed developmental stages. A probe without cDNA was used as the negative control (ntc). A rabbit liver cDNA sample was used as the positive control (+). The internal control was the expression of GAPDH in all probes. bp, basepairs.

these changes are not in accordance with the composition in oviductal and uterine fluid (Khandoker *et al.*, 1998). Taking this together with the observations in our study, we propose that the embryo has a specific preference to take up individual FAs, thus modulating their own fatty acid composition independently from the maternal supply.

We have shown that the lipid content and metabolism of rabbit blastocysts changes in a diabetic environment (Schindler *et al.*, 2014). In this study, we demonstrated that the FA composition in EB and TB are affected differently by maternal insulin-dependent diabetes mellitus, with an increase in SFAs and a decrease in n-3 PUFAs, especially in the EB. In eukaryotic cells and even in embryos, FAs are highly diverse in terms of their metabolic and functional properties. The relationship between FA metabolism and embryo differentiation and implantation was summarized by Revelli *et al.* (2009) and Dunning *et al.* (2014). It is known that changes in the FA composition affect embryonic metabolism and further development in a cell lineage-specific manner. PA and SA decrease rates of fertilisation, cleavage and blastocyst formation by regulating the genes responsible for stress and apoptosis (Jungheim *et al.*, 2011; McKeegan and Sturmey, 2011; Valckx *et al.*, 2014). OA compensates the negative effects of PA and moreover, elevated levels of OAs can even improve post-fertilisation developmental competence and implantation (Aardema *et al.*, 2011, Valckx *et al.*, 2014; Yang *et al.*, 2017). We observed increased levels of OA and decreased levels of PA and SA in the TB, while in the EB, PA and SA were found to be increased and OA levels were not affected. These results indicate that the EB could be affected by oxidative stress and apoptosis. In a previous study, we described embryo development capacities and showed that apoptosis increases in the EB from diabetic rabbits (Ramin *et al.*, 2010). Controversy exists regarding the role of LA for preimplantation embryo development. A positive impact of LA on the fertility rate was documented in humans (Shaaker *et al.*, 2012). However, studies in ovine and bovine models have argued that high levels of LA negatively affect oocyte maturation and developmental competence (Renaville *et al.*, 2010; Amini *et al.*, 2016; Roura *et al.*, 2018). In humans, ALA exerts damaging effects on the reproductive system, decreasing the chance of pregnancy after IVF (Jungheim *et al.*, 2011). Both LA and ALA were found to be increased in all compartments of the rabbit blastocysts due to maternal diabetes mellitus. This may contribute to the adverse effect of a diabetic pregnancy. Haggerty and co-workers found higher levels of DHA in later stages of preimplantation embryo development (Haggarty *et al.*, 2006), indicating that this FA is important for the later phase of preimplantation development. We observed an excessive reduction in DHA in EB and BF from diabetic rabbits. This correlates with studies in humans, where a diabetic pregnancy led to lower DHA in embryonic and foetal tissues (reviewed by Léveillé *et al.* (2018) and Judge *et al.* (2016)). In a bovine

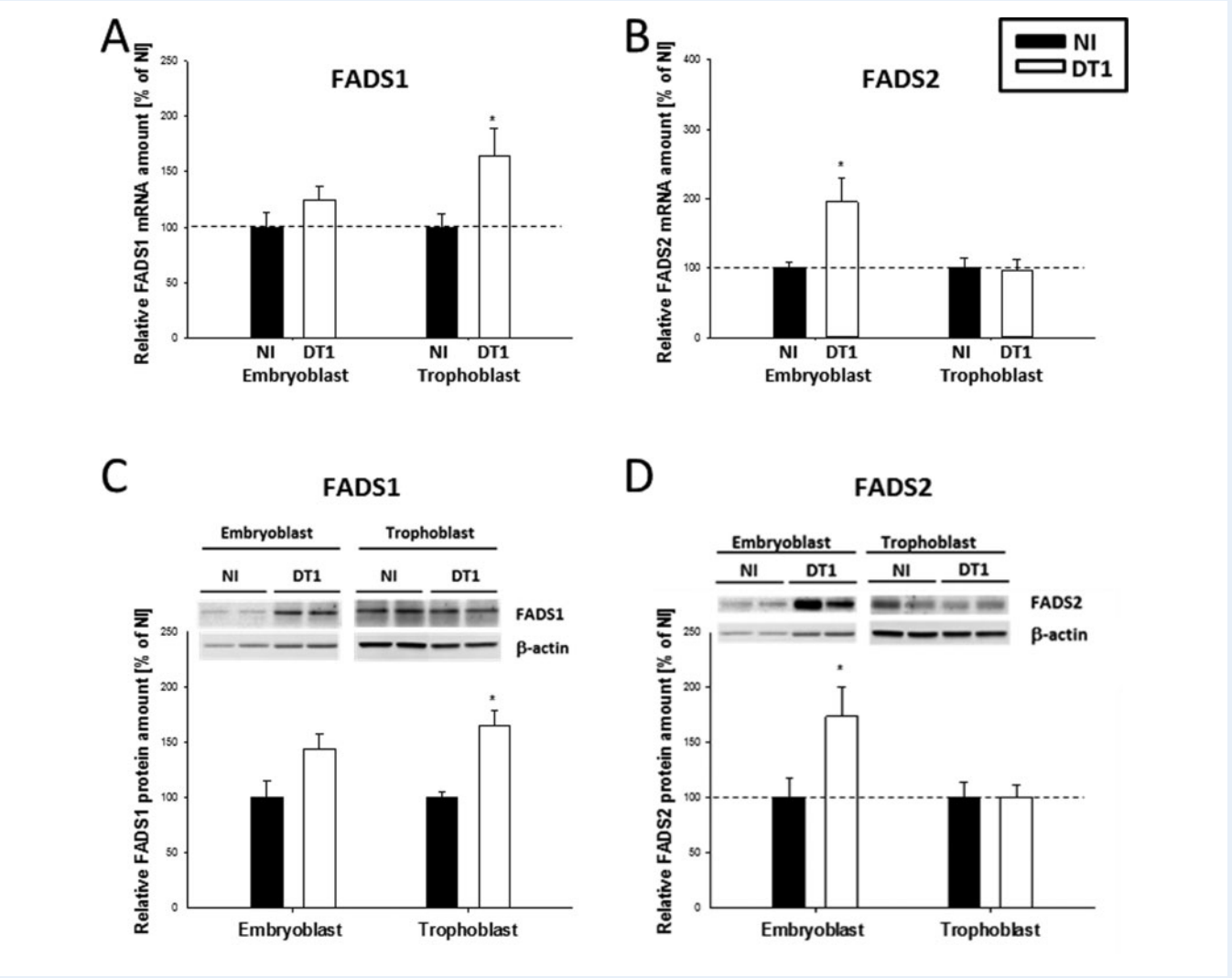

Figure 2. FADS1 and FADS2 expression by RT-qPCR and western blot in blastocysts from diabetic rabbits. Transcription levels (RT-qPCR) of FADS1 (**A**) and FADS2 (**B**) in blastocysts from normoinsulinaemic (NI, black bars) and diabetic rabbits (DT1, white bars) are given. FADS1 and FADS2 mRNA levels were elevated in EB and TB, respectively (*$P < 0.05$). The protein expression (western blot) of FADS1 (**C**) and FADS2 (**D**) was quantified in 6-day-old blastocysts from DT1 (white bars) and NI (black bars). For western blot quantification, samples from at least three independent experiments with eight or more blastocysts per samples were analysed ($n \geq 3$, $n \geq 8$). A representative western blot is shown (C and D). Protein was detected using Luminata Forte (Milipore) imaged with Chemi Doc ImageLab Software. The data presented in the submitted manuscript are the mean ± SEM of two separate blots. The relative amounts of FADS1 and FADS2 are shown in diagrams after normalization for the levels of β-actin. All values are expressed as relative amounts (mean + SEM) in % of non-diabetic controls ($n \geq 8$,*$P < 0.05$).

model, DHA affects oocyte developmental competence by regulating lipid metabolism gene expression (Oseikria *et al.*, 2016). Therefore, we believe that changes in DHA due to maternal diabetes mellitus may contribute to early diabetic embryopathy.

Based on the work from Childs *et al.* (2008), it is known that external factors such as diet can affect the FA composition of the genital tract. In the present study, we showed that experimentally-induced diabetes mellitus led to reduced total amount of SFAs and MUFAs in maternal plasma, while the total amount of PUFAs increased. In detail, no changes were observed for PA and OA, whereas SA, DPA and DHA decreased and LA and ALA increased. Changes in the plasma FA profile are known from humans, with special attention to the reduction in DHA level (Taschereau-Charron *et al.*, 2018).

The degree of unsaturation and the length of the FAs are modulated by desaturation and elongation enzymes (Fig. 4). All of the enzymes that we analysed were expressed in the rabbit preimplantation embryo from Day 3 p.c. and onward and in both blastocysts compartments, EB and TB. The expression of FA desaturases and elongases has been described in other species, too. In sheep d7 blastocysts, FADS1, FADS2 and ELOVL5 were expressed (Hughes *et al.*, 2011), and during bovine preimplantation embryo development ELOVL5 and ELOVL6 were expressed, with the most prominent expression during the

Table V Relative desaturases activity index in embryoblast (EB) and trophoblast (TB) cells of normoinsulinaemic (NI) and experimental insulin-dependent diabetic (DT1) rabbits.

Index of enzyme activity		Embryoblast (EB)			Trophoblast (TB)		
		NI	DT1	*P*-value	NI	DT1	*P*-value
Δ5-desaturase activity	Relative activity (ratio 20:4n-6/20:3n-6)	$1.14_{\pm 0.824}$	$0.75_{\pm 0.361}$	*0.627*	$4.88_{\pm 0.502}$	$3.76_{\pm 0.330}$	*0.062*
Δ6-desaturase activity	Relative activity (ratio 18:3n-6/18:2n-6)	$0.19_{\pm 0.024}$	$0.24_{\pm 0.033}$	*0.190*	$0.05_{\pm 0.007}$	$0.01_{\pm 0.001}$	*<0.001**

Activity was calculated as ratio of AA (20:4n-6) to dihomo-γ-linolenic acid (20:3n-6) and γ-linolenic acid (18:3n-6) to linoleic acid (18:2n-6) for Δ5 and Δ6 desaturase, respectively. Results are expressed as $Mean_{\pm SEM}$ (from n = 20 individual samples). Statistical significances are indicated as follows * $p < 0.05$.

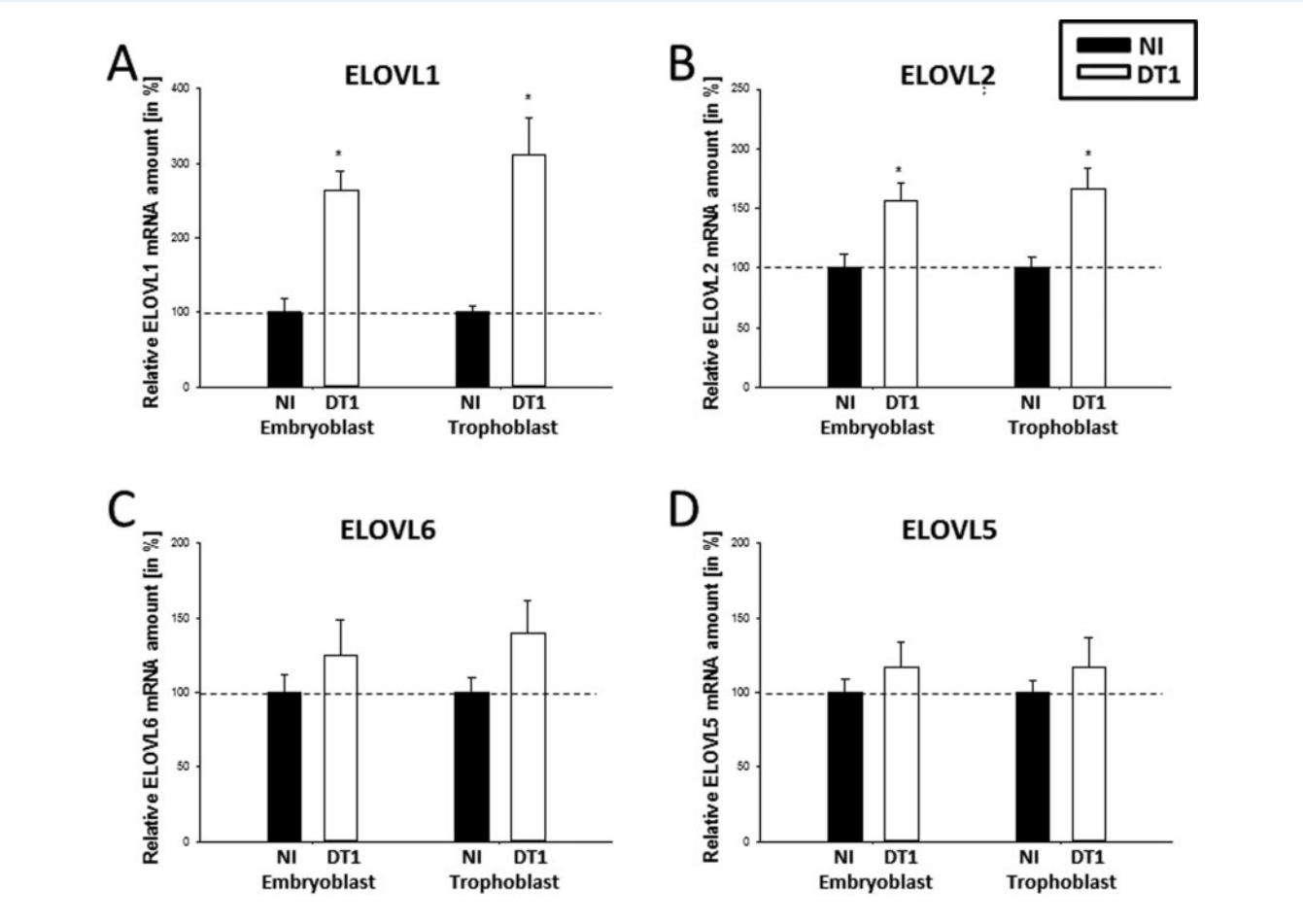

Figure 3. ELOVL1, ELOVL2, ELOVL5 and ELOVL6 expression by RT-qPCR in blastocysts from diabetic rabbits. Transcription analyses (RT-qPCR) of ELOVL1 (**A**), ELOVL2 (**B**), ELOVL5 (**C**) and ELVOL6 (**D**) in blastocysts from normoinsulinaemic (NI, black bars) and diabetic rabbits (DT1, white bars) are given. The results are shown as mean + SEM and transcription in normoinsulinaemic blastocysts was set 100%. The transcription of ELOVL1 and ELVOL2 was increased in embryoblast and trophoblast, while ELOVL5 and ELOVL6 was not changed by maternal diabetes (*$P < 0.05$).

morulae stage (Sudano *et al.*, 2016). Mainly *in vitro* studies have shown how the expression of desaturases and elongases can be affected by the surrounding milieu. In sheep and bovine embryos, FA supplementation affects embryonic of FADS2 expression (Darwich *et al.*, 2010; Hughes *et al.*, 2011). FADS1, FADS2 and ELOVL6 activity and expression is regulated by a wide range of external factors which

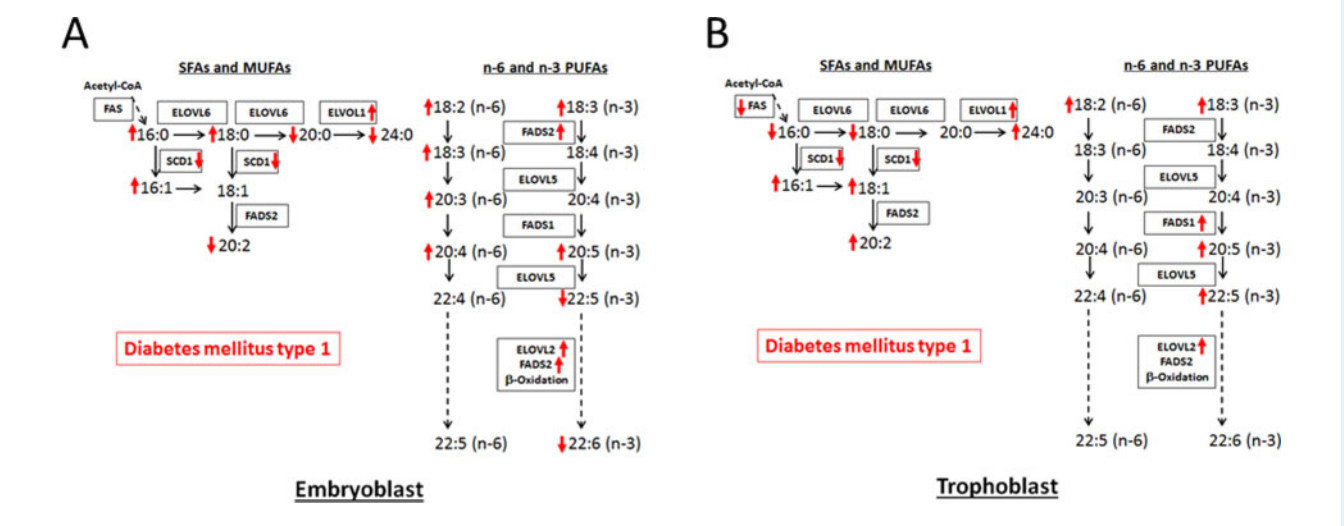

Figure 4. Adaptation in FA metabolism of rabbit blastocysts to maternal diabetes mellitus type 1. Diabetic changes in embryoblast (A) and trophoblast (B) are schematically summarized and indicated by red arrows (up: increase, down: decrease). (A) In the EB, FAD2, ELOVL1 and ELOVL2 increased, accompanied by higher levels of saturated fatty acid (SFA), palmitic acid (PA) (16:0) and stearic acid (SA) (18:0) and n-6 PUFAs such as linoleic acid (LA) (18:2n-6) and arachidonic acid (AA) (20:4n-6). Long chain n-3 PUFAs, such as docosapentaenic acid (DPA) (22:5n-3) and docosahexaenoic acid (DHA) (22:6 n-3) were greatly decreased. (B) In the TB FAD2, ELOVL1 and ELOVL2 increased. FA synthase (FAS) was reduced in the TB (Schindler, unpublished), leading to lower levels of SFA, such as PA (16:0) and SA (18:0). Oleic acid (OA) (18:1cis-9) was increased, as well as LA (18:2n-6) and ALA (18:3n-3).

are primarily mediated by sterol regulatory element-binding protein-1c (SREBP1c) (Wang *et al.*, 2006). Insulin is the main regulator of SREBP1c activity (Czech *et al.*, 2013). Streptozotocin-induced diabetes in mice led to a diminished amount of nuclear SREBP1, accompanied by a downregulation of ELOVL6 and Δ9-desaturase (SCD1) (Wang *et al.*, 2006). In mouse and ovine tissues, insulin is able to regulate SCD1, FADS1, and FADS2 (Brenner, 2003; Daniel *et al.*, 2004). We have shown that experimentally-induced diabetes mellitus reduces the protein amount of SCD1 in EB and TB and it causes lower fatty acid synthase (FAS) protein levels in TB (Schindler, unpublished). The reduced trophoblastic FAS expression, may therefore, explain the lower PA and SA levels observed in the TB from diabetic rabbits. In the current study, FADS1 expression was increased in TB, while the FADS1 activity index (ratio of 20:4n-6 vs 20:3n-6) was reduced in both cell compartments by 25–30%. FADS2 expression was increased in EB cells from diabetic rabbits. The activity index of the FADS2, however, was slightly increased in EB, while in TB the FADS2 activity was doubled. As previously mentioned, both FADS1 and FADS2 are regulated by SREBP1. In a recent study, we showed that blastocysts from diabetic rabbits have a higher amount of nuclear SREBP1 (nSREBP1, the active form of SREBP1) (Schindler *et al.*, 2014), indicating that the higher amounts of FADS1 and FADS2 are due to the induced activation of SREBP1. In contrast, Wang and co-workers (Wang *et al.*, 2006) showed that from the elongation enzymes only ELOVL6 was affected in the diabetic model. We measured increased ELOVL1 and ELOVL2 amounts in EB and TB from diabetic rabbits. These differences may be due to alternative regulation by other transcription factors, such as liver X receptor (LXR) or peroxisome proliferator-activated receptors α, which are altered under diabetic conditions (Wang *et al.*, 2006, Schindler *et al.*, 2017).

Taken together, over the last decade a large number of studies have suggested that the physiological roles of FAs during pregnancy extend beyond that of an energy source and include the regulation of cell metabolism, cell signalling and development. Exposure to maternal diabetes mellitus during the first days of development affects the FA composition in embryonic tissues and induced changes in elongation and desaturation enzymes (summarized in Fig. 4). Whether changes in elongation and desaturation enzymes are responsible for the altered external FA composition in rabbit embryos from diabetic mothers or whether altered FAs supply and uptake influence expression of elongases and desaturases has to be elucidated in further studies. However, the current study clearly showed that maternal diabetes mellitus affects embryonic FA composition and metabolism in a cell lineage-specific manner, and the obtained results advance our understanding of the pathology of periconceptional diabetes.

Supplementary data

Supplementary data are available at *Molecular Human Reproduction* online.

Data availability

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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Authors' roles

All authors have read and agree to the published version of the manuscript. The authors contributed to the manuscript as follow: conceptualization, M.S. and A.N.S.; methodology, M.S., D.D., M.P., K.G. and T.S.; formal analysis, D.D., G.N. and M.S.; investigation, M.S.; data curation, M.S.; original draft preparation, M.S.; review and editing, D.D., T.S. and A.NS.; visualization, M.S.; supervision, D.D. and M.S.; project administration, M.S.; funding acquisition, M.S., D.D. and A.N.S.

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Conflict of interest

The authors have no conflicts of interest to disclose.

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